Eco. Env. & Cons. 29 (May Suppl. Issue) : 2023; pp. (S492-S496)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i03s.084

Genetic variation within the melatonin receptor 1C (MTNR1C) gene in the native chicken 'Zo-ar' of Mizoram, India

Princelina Bora[1], T.C. Tolenkhomba[2], Purabi Kaushik[3]* and N. Shyamsana Singh[4]

College of Veterinary Science, Assam Agricultural University, Khanapara, Assam, India
[1]Department of Animal Genetics and Breeding, College of Veterinary Science, Assam Agricultural University, Khanapara, Assam, India
[2]Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India
[3]Department of LFC (Animal Genetics and Breeding), College of Veterinary Science, Assam Agricultural University, Khanapara, Assam, India
[4]Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

(Received 18 December, 2022; Accepted 14 February, 2023)

ABSTRACT

The study has been aimed to uncover the presence of polymorphism within the MTNR1C gene in native chicken population, 'Zo-ar' of the Mizoram state of India and to calculate the allelic and genotypic frequency to screen the variation present within the population with respect to the MTNR1C gene which is associated with various egg production traits in poultry. DNA extraction has been done with the help of blood samples collected from a total of 50 randomly chosen 'Zo-ar' chicken irrespective of age or sex from different regions of Mizoram. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay has been used for the detection of SNPs within the MTNR1C gene. Two genotypes AG (0.34) and GG (0.66) have been detected for the MTNR1C gene in the 'Zo-ar' chicken population while the AA (0.00) genotype was found to be completely absent in the present study. The G allele (0.83) was found to be predominantly present in the populations. Further, the population was found to be conforming to the Hardy-Weinberg Equilibrium with respect to the MTNR1C locus. From the present study the presence of variation within the MTNR1C locus was seen in the 'Zo-ar' chicken of Mizoram. This indicates the possibility of genetic improvement in egg production traits by using proper selection methods and formulating appropriate mating systems.

Key words : *MTNR1C gene, 'Zo-ar' chicken, PCR-RFLP, SNP, Variation, Genetic improvement*

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized in the pineal gland having an important effect on various physiological and reproductive processes along with controlling the circadian rhythm of the body (Kharwar *et al.,* 2011; Trivedi and Kumar, 2014). Out of the three melatonin receptors, MTN1A, MTNR1B and MTNR1C the latter is found only in birds and amphibians but not

([1]Research Scholar, [2] Assistant Prof.(SG), [3]Assistant Prof., [4]Associate Prof. and Head)

in mammals (Sundaresan *et al*., 2009). The melatonin receptor 1C, MTNR1C (Mel1C or MT3) is a G-protein coupled receptor that binds melatonin. In poultry the MTNR1C has been found to be regulating hibernation, feeding pattern, thermoregulation, circardian rhythm along with neuroendocrine functions (Courtillot *et al.,* 2010). The MTNR1C gene was also found to be present in granulose layer of cells, ovaries and ovarian follicular fluid of birds suggesting a possible role of melatonin and its receptor genes on female reproductive process in domestic chicken (Sundaresan *et al*., 2009). Also, the MTNR1C gene and its polymorphisms has been reported to be associated with age at first egg (AFE) and egg numbers at 300 days (NE 300d) in chicken suggesting it as a candidate gene for economic traits (Li *et al.,* 2013; Feng *et al*., 2018; Tenzin *et al*., 2020).

The local chicken 'Zo-ar' of Mizoram are medium sized birds (Lalhlimpuia, 2020) which are is scavenging in nature. They are generally reared for both games as well as table purpose by the local dwellers production (Mayengbam *et al.,* 2017). The body weight gain of this local breed of chicken was found to be superior to the body weights of breeds like Naked Neck and Frizzle fowl. Whereas, the Hen Day Egg Production (HDPE) percentage of these native chickens was low, which have been thought to be due to the persistent broodiness that effects the overall egg production (Mayengbam *et al.,* 2017). Moreover, the average annual egg production of these chickens was also found to be low, about 86.49 per year (BAHS, 2019). However, very little work has been carried out to exploit the genetic potential of these native fowls. Genetic selection when done only for increasing the production capacity can result in the reduction of the innate ability of disease resistance (Zekarias *et al*., 2002). Again, multitrait selection in order to improve production without compromising fitness which is an inborn advantage of the native population is difficult to achieve with traditional methods like that of phenotypic selection. Thus, if attention is given on finding out information on the genetic constitution with respect to genes related to egg production, body weight gain and other economic traits, an overall improvement can be achieved with respect to its increase in economic importance.

Therefore, the main aim of this study has been focused on the detection of polymorphism in the MTNR1C gene in the local chicken 'Zo-ar' of Mizoram. Observation of the genetic variation present within the population and distribution pattern of the different genotypes along with the available amount of heterozygosity has been done which will help in determining the possibility of genetic improvement in the population regarding egg production and egg numbers.

Materials and Methods

Ethical approval

The methods and techniques used in the study follows the standard rule of animal ethics in the country and have been approved by the Institutional Animal Ethics Committee (IAEC) of India. The reference number for approval is CVSC/CAU/IAEC/19-20/P-21.

Chickens and Blood samples

Blood samples (1 ml) were collected randomly from a total of 50 unrrelated, randomly chosen 'Zo-ar' chicken of Mizoram, India. The chickens were taken from backyard reared flocks of the households in different districts of the state *viz*. Kolasib, Mamit and Aizawl districts. The samples were collected aseptically from the wing vein in EDTA vials and kept immediately in ice pack containers. Thereafter, brought directly to the laboratory and stored at -20 ^{o}C until further use.

Genomic DNA isolation, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

DNA was done from the samples using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) following the attached protocols that are provided with the kit. Quantity and quality of the isolated DNA were done using a Nanodrop Spectrophotometer (Thermo Scientific, USA) and electrophoresis was done on 0.75% agarose gel for checking any shearing of the extracted DNA. The optical density ratio (OD_{260}/OD_{280}) of the DNA samples was checked and only those having an OD_{260}/OD_{280} ratio of 1.7 to 1.9 were subjected to further analysis for molecular and quantitative data.

A PCR mixture of 25 µL was prepared for amplification consisting of 10X PCR buffer, 200 µM of each dNTPs, 5 pM of each forward and reverse primers, 2U Taq DNA polymerase, 2mM of $Mgcl_2$ and 60 ng of extracted genomic DNA. Forward primer 5'- ggtgtatccgtatcctctaa -3' and reverse

primer 5'- gacagtgggacaatgaagt -3' (Li *et al.*, 2013) were used. PCR amplification of the MTNR1C gene was done using the following cycles : Pre Denaturation at 95 ºC for 5 min, followed by 30 cycles of denaturation at 95 ºC for 45 sec, annealing at 61 ºC for 60 sec, elongation at 72 ºC for 45 sec and post extension at 72 ºC for 5 min. The obtained amplicon size was 372 bp. The PCR products were then subjected to RE digestion using *Mbo*I restriction endonuclease enzyme by incubating at 37ºC for overnight. The restriction enzyme digested products were then separated for visualising the bands in 2% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide and observed under UV trans-illuminator. Photographs of the gel were taken using Gel Doc system for further interpretation. The primers and restriction enzymes used for PCR-RFLP analysis have been mentioned in Table 1.

Statistical Analysis

The allele frequencies, genotypic frequencies were estimated using the POPGENE 32 software (Yeh *et al.*, 1997) for population genetics analysis following the given formulae:

$$\text{Genotype frequency} = \frac{D}{N}$$

$$\text{Gene frequency} = \frac{2D + H}{2N}$$

Where,

D = Number of individuals having the particular genotype in the population

H = Number of the heterozygous genotype in the population

N = Total number of the individuals in the population

The tests for variation from Hardy Weinberg equilibrium were performed using exact probability (P-values) tests provided in POPGENE 32 version from the genotypic frequencies obtained.

The observed and expected heterozygosity were also calculated using the POPGENE 32 software.

The observed heterozygosity (H_o) has been calculated as the actual percentage of heterozygosity occurring in the sample population.

$$Ho = \frac{number\ of\ heterozygotes}{total\ number\ of\ samples} \times 100$$

The expected heterozygosity or genetic diversity was measured (Nei, 1972) by the formula mentioned below,

$He=1-\Sigma P^2_i$ (Where, P_i is the frequency of i^{th} allele).

Results

The digestion of MTNR1C gene PCR amplified fragment of 372 bp with the restriction endonuclease enzyme *Mbo*I showed polymorphism in the locus, by yielding only two different genotypes out of three expected genotypes. The two genotypes were AG (372, 333 and 39 bp) and GG (333 and 39 bp). However, the genotype AA was completely absent in this Mizoram local chicken population. Both the genotypes AG and GG are shown in Figure 1. The

Table 1. Gene, PCR amplicon size, primer sequence, annealing temperature for PCR and restriction endonuclease (RE) enzyme used for RFLP analysis.

Gene	Primer sequence (5´ - 3´)		T^A (°C)	Product size (bp)	RE	Incubation
MTNR1C	F	GGTGTATCCGTATCCTCTAA	49	372	*Mbo*I	37ºC overnight
	R	GACAGTGGGACAATGAAGT				

Table 2. Genotypic frequency distribution and heterozygosity of MTNR1C gene

Parameters		'Zo-ar' chicken (n=50)
Genotypic Frequencies	AA	0.00 (0)
	AG	0.34 (17)
	GG	0.66 (33)
χ^2 value	1.96^{NS}	
Observed Heterozygosity	0.34	
Expected Heterozygosity	0.28	

n = Number of animals; NS = Not significant; the figures in parenthesis are the number of animals

genotype GG was more commonly found with a frequency of 0.66 in the population. On the other hand the AG genotype had a frequency of 0.34. Moreover the population was also seen to be in Hardy-Weinberg equilibrium with respect to the MTNR1C locus (Table 2). The G allele was most extensively found with a frequency of 0.83 while the A allele was with a low frequency of 0.17, which showed the A allele was very scarcely present in the Mizo-local chicken population (Table 3).

Table 3. Allele frequency of MTNR1C locus

Locus	Allele	Allelic frequency
MTNR1C	A	0.17
	G	0.83

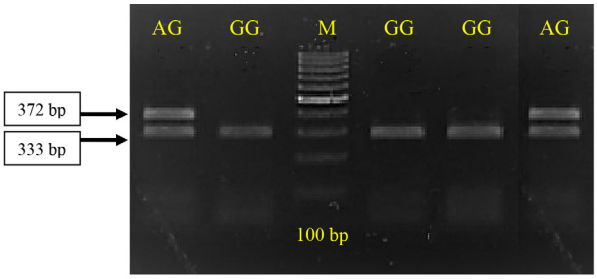

Fig. 1. Genotypes of MTNR1C gene digested with RE *Mbo*l in 2% agarose gel Lane M: 100 bp gene ruler

The observed heterozygosity (0.34) was found to be higher than the expected heterozygosity (0.28) in the studieed population.

Disscussion

Similar reports of polymorphisms were found to be present in the MTNR1C locus for different breeds of chicken like Noi chicken of Vietnam (Vu and Ngu, 2016), Earlang Mountain chicken (Li *et al*., 2013), Pradu Hang Dam chicken, Chee chickens of Thailand (Tenzin *et al*., 2020) and Shaoxing ducks (Feng *et al*., 2018).

The absence of AA genotype and abundance of GG genotype in our findings were in close agreement with the results observed in Noi chicken of Vietnam (Vu and Ngu, 2016) where the AA genotype was very rarely found (0.06) and the GG genotype was most abundant (0.54) among the population which is similar to our findings. Likewise, the AA genotype was found to be having the lowest frequency in both Chee (0.08) and Pradu Hang Dam (0.06) chicken of Thailand (Tenzin *et al*., 2020). On the other hand, in Earlang Mountain chicken (Li *et al*., 2013), all the three genotypes were reported where the heterozygote genotype AG was found to have a higher frequency (0.64), followed by the GG (0.19) and AA (0.17) genotypes. The predominance of the G allele in our findings was found to be in accordance with that in Noi chicken of Vietnam (Vu and Ngu, 2016) where the G allele frequency was found to be 0.74. In Earlang Mountain chicken too the G allele (0.51) was found to be the major allele (Li *et al*., 2013). Likewise, in Pradu Hang Dam chicken (0.73) and Chee chicken (0.80) of Thailand showed similar results of G allele predominance (Tenzin *et al*., 2020).

In Earlang Mountain chicken (Li *et al*., 2013) significant correlation of the MTNR1C genotypes was found with production traits like age at first egg (AFE) and total egg numbers (EN). The heterozygotes AG had shorter AFE and higher EN as compared to the other two genotypes. In two Thai native chicken breeds namely, Pradu Hang Dam chicken and Chee chickens (Tenzin *et al*., 2020), it was observed the significant associations of MTNR1C genotype (AA) with egg production traits. These finding suggested that AG and AA genotype are responsible for improved performance for economic traits in chicken.

In the present study, presence of AG genotype in medium frequency (0.34) revealed that genomic selection may be useful for genetic improvement of economic traits in this native chicken of Mizoram.

Conclusion

The present analysis may be concluded by asserting that variation within the MTNR1C locus is present in the 'Zo-ar' chicken population of Mizoram. Moderate amount of heterozygosity and genetic variability is present indicating the possibility of genetic improvement. The absence of the AA genotype can be confirmed by performing future studies. The population was found to be in accordance with the Hardy-Weinberg equilibrium suggesting the absence of selection with respect to egg production traits and also absence of migration in the breeding tract. Thus, the knowledge generated on the allelic and genotypic distribution pattern in the native chicken population can be used for future genetic improvement of the population.

Acknowledgement

The authors of the present study are very thankful to Central Agricultural University, Imphal, Manipur and the whole staff of Department of Animal Genetics and Breeding, C.V.Sc. and A.H., Selesih, Aizawl, Mizoram for providing financial support and technical assistance respectively during the course of study.

References

BAHS (Basic Animal Husbandry Statistics), 2019. Government of India, Ministry of Fisheries, Animal Husbandry and Dairying, DAHD, Krishi Bhawan, New Delhi.

Courtillot, C., Chakhtoura, Z., Bogorad, R., Genestie, C., Bernichtein, S. and Badachi, Y 2010. Characterization of two consecutively active prolactin receptor variants in a cohort of 95 women with multiple breast fibroadenomas. *J. Clin. Endocrinol. Metab*. 95(1): 271 – 279.

Feng, P., Zhao, W., Xie, Q., Zeng, T., Lu, L. and Yang, L. 2018. Polymorphisms of melatonin receptor genes and their associations with egg production traits in Shaoxing duck. Asian-Australas. *J. Anim. Sci*. 21(10): 1535-1541.

Haunshi, S. and Doley, S. 2011. Performance of Native Chickens of Mizoram under Intensive System of Rearing. *Indian Vet. J*. 88(3): 45-47.

Kharwar, R.K. and Haldar, C. 2011. Anatomical and histological profile of bronchus- associated lymphoid tissue and localization of melatonin receptor types (Mel1a and Mel1b) in the lung associated immune system of the tropical bird, *Peridicula asiatica*. *Acta. Histochem*. 113(3): 333-339.

Lalhlimpuia, C. 2020. *Phenotypic characterization of Local Chicken of Mizoram in its Home tract*. M.V.Sc. Thesis, submitted to Central Agricultural University, Imphal, Manipur

Li, D.Y., Zhang, L., Smith, D.G., Xu, H.L., Liu, Y.P., Zhao, X.L., Wang, Y. and Zhu, Q. 2013. Genetic effects of melatonin receptor gene on chicken reproductive traits. *Czech. J. Anim. Sci*. 58(2): 58-64.

Mayengbam, P., Tolenkhomba, T.C. and Ali, M.A. 2017. A Brief Biological Note on Semi-wild Indigenous Chicken 'Sikhar' of Mizoram, India. *Int. J. Livest. Res*. **7**(8): 146-152.

Nei, M. 1972. Genetic distance between populations. *Am. Nat.* 106 : 283-291.

Sundaresan, N.R., Marcus-Leo, M.D., Subramani, J., Anish, D., Sudhagar, M., Ahmed, K.A., Saxena, M., Tyagi, J.S., Sastry, K.V. and Saxena, V.K. 2009. Expression analysis of melatonin receptor subtypes in the ovary of domestic chicken. *Vet. Res. Commun*. 33(1): 49-56.

Tenzin, J., Chankitisakul, V. and Boonkum, W. 2020. Association of polymorphisms of physiological candidate genes with phenotype and estimated breeding values of reproductive and growth traits in Thai indigenous chickens. *Genet. Mol. Res*. 19(1): 1-19.

Trivedi, A.K. and Kumar, V. 2014. Melatonin: an internal signal for daily and seasonal timing. *Indian J. Exp. Biol*. 52(5): 425-437.

Vu, C.T. and Ngu, N.T. 2016. Single nucleotide polymorphisms in candidate genes associated with egg production traits in native Noi chicken of Vietnam. *Int. J. Plant Anim. Environ. Sci.* 6(1): 162-169.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H. and Mao, J.X. 1997. POPGENE the user friendly shareware for population genetic analysis. University of Alberta, Canada.

Zekarias, B., TerHuurne, A.A., Landman, W.J., Rebel, J.M., Pol, J.M. and Gruys, E. 2002. Immunological basis of differences in disease resistance in the chicken. *Vet. Res.* 33(2): 109-125.